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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
P045461PCT RDU/do		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL2004/000420	14.06.2004	13.06.2003

International Patent Classification (IPC) or national classification and IPC
A61K35/74, A23L1/30, C12N1/20, A61P1/00

Applicant
N.V. NUTRICIA et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11.04.2005	13.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Bayrak, S Telephone No. +31 70 340-3263

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IAP20 Rec'd PCT/PTO 08 DEC 2005

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NL2004/000420

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-28	as originally filed
Sequence listings part of the description, Pages	
29-31	as originally filed
Claims, Numbers	
1-16	received on 11.04.2005 with letter of 11.04.2005
Drawings, Sheets	
1/1	as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-15
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-11, 13-16 (all partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-11, 13-16 (all partially) (see separate sheet)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.

PCT/NL2004/000420

Re Item III.

1. Present claims 1-6, 8-11, 13-16 relate to a composition defined by reference to a desirable characteristic or property, namely "pediocin-producing pediococci", "..., wherein the pediococci are characterised by a survival rate..", "pediococci are isolated from human faeces", "bacteriocin produced from pediococci..", "antibiotic derived from quinolones". The claims cover all compositions having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compositions. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the composition by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

2. Claims 1-10 relate to the use of a pharmaceutical preparation for the prophylaxis or treatment of diseases which are functionally defined "for inhibiting pathogenic strains in the gastrointestinal tract", or "secondary disorders being preferably selected from water disturbances, mineral balance disturbances, malnutrition, dysfunctioning of tissues, dysfunctioning of organs, dysfunctioning of organism, and mixtures thereof", which encompass a multitude of different diseases. The claims thus cover a rather large number of diseases, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of diseases. Consequently, the claims lack support and the application lacks disclosure. Independent of the above reasoning, the claims 1-10 also lack clarity because it is not fully possible to determine the diseases for which protection might legitimately be sought (Article 6 PCT).

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the use of P. acidilactici or P. pentosaceus in the treatment of bacterial infection of the gastrointestinal tract and diarrhoea; and with due respect to the general idea of the invention.

No opinion will be given in respect of subject matter which is not covered by the

search report (Rule 66.1(e)PCT)

Re Item V.

The following documents are referred to in this communication:

D1: XP-002241735
D2: WO9729645

1 **NOVELTY (Art. 33(2) PCT)**

1.1 The subject matter of the present application, **insofar as clear**, is new with regard to D1-2:

1. Document D1 discloses the use of pediococci for the manufacture of a composition for inhibiting pathogenic strains in the gastrointestinal tract (probiotic). The P. acidilactici strain P-2 disclosed in D1 however has a survival rate of 49%, according to the Survival Rate Test in the application (see example 1 (table 3) of the application in which several strains of pediococci are compared with regard to the survival rate in stomach medium (pH3)). Although no figures are shown for Pediococcus pentosaceus (FF), document D1 states that "P. acidilactici strain P-2 had the strongest capacity for surviving acidic conditions and 0.3% bile salts" (cf. page 299, left column: "Conclusion"), thus indicating that the survival rate Pediococcus pentosaceus (FF) is even less than that of P-2. Moreover, at pH 4 the count of viable bacteria in all investigated strains was less than 5 log cfu/ml (cf. page 299, left column, line 1-6)). **Document D1 does not disclose the Pediococus acidilactici strain LMG P-21927.**

2. Document D2 discloses a health composition comprising living organisms, among which *P. pentosaceus*, *P. acidilactici* are enlisted, and bacteriocin, among which is pediocin mentioned, as animal feed supplement or composition for preventing pathogenic bacteria from adhering to the mucous membrane surfaces, restoring bacterial flora, preventing or treating infections and diarrhoea in the gut (cf. whole document). D2 does not specifically disclose a pediocin- producing

pediococcus having a high survival rate in the conditions of the stomach and the gastrointestinal tract. **Document D2 does not disclose the Pediococcus acidilactici strain LMG P-21927.**

Therefore the subject matter of claims 1-16, insofar as clear, is new (Article 33(2) PCT).

2 INVENTIVE STEP (Art. 33(3) PCT)

2.1 D1, which is the closest prior art, suggests the use of probiotic lactic acid bacteria with good survival capacity in the stomach and the gastrointestinal tract for the therapy of diseases of the gastrointestinal tract associated with pathogenic bacteria. The Pediococcus acidilactici (P2) is disclosed in D1 as the strain having the best survival capacities under acidic conditions and at the higher concentration of bile salts (a survival rate of 49% according to the Survival Rate Test in the application). From this the subject mater of the present application differs in that pediocin-producing pediococci are used which have a survival rate of at least 80% according to the survival rate test of the application.

Thus the problem to be solved by the present application can be considered as provision of a pediocin-producing pediococcus strain(s) with high survival capacities (>80% as defined by the survival rate test) in the stomach and the gastrointestinal tract.

The solution proposed is the use of the pediocin-producing pediococcus strain LMG P-21927 isolated from human faeces which ha a high survival rate (at least 80%) in the conditions of the stomach and the gastrointestinal tract.

Although the use of probiotic lactic acid bacterial strains with good survival capacity under the conditions of the stomach and the gastrointestinal tract are suggested in D1, the use the pediocin producing Pediococcus acidilactici strain LMG P-21927 with a survival rate of at least 80% is not suggested in D1. Document D2 discloses a health composition comprising living organisms, among which *P. pentosaceus*, *P. acidilactici* are enlisted, and bacteriocins (pediocin A, nisin, pediocin

AcH and others) as animal feed supplement or composition for preventing pathogenic bacteria from adhering to the mucous membrane surfaces, restoring bacterial flora, preventing or treating infections and diarrhoea in the gut (cf. whole document).

However, D2 does not disclose a pediocin-producing pediococcus having a good survival rate in stomach and the GI tract, as it focuses rather on the health effect of the total composition of strains, organic acids and bacteriocins. Moreover, the bacteriocins could also include nisin, which is detrimental also to beneficial strains in the intestines (see page 2, line 1-4 of the application).Thus D2 does not suggest the use the Pediococcus acidilactici strain LMG P-21927 which has a survival rate of at least 80%.

Therefore, in view of document D1-2, the subject-matter of the present application, **insofar as clear**, appears to involve an inventive step in the sense of Art. 33(3) PCT.

3 **INDUSTRIAL APPLICABILITY (Article 33(4) PCT)**

Claims 1-16, insofar as clear, fulfil the requirements of (Article 33(4) PCT).

AMENDED CLAIMS

1. Use of pediocin-producing pediococci for the manufacture of a composition for inhibiting the growth of pathogenic strains in the gastrointestinal tract, wherein the pediococci are characterised by a survival rate as per the Survival Rate Test defined herein of at least 80 %.

2. Use according to claim 1, wherein the survival rate is at least 90 %.

3. Use according to claim 1 or 2, wherein the pediococci are isolated from human faeces.

4. Use of pediocin-producing pediococci for the manufacture of a composition for inhibiting the growth of pathogenic bacteria in the gastrointestinal tract, wherein the pediococci are isolated from human faeces.

5. Use according to any one of the preceding claims for preventing and/or treating infections by pathogenic bacteria in the gastrointestinal tract, diarrhoea, and/or secondary disorders associated herewith.

6. Use according to claim 5, wherein the secondary disorders comprise water disturbances, mineral balance disturbances, malnutrition, dysfunctioning of tissues, dysfunctioning of organs, dysfunctioning of organism, and combinations thereof.

7. Use according to any one of the preceding claims, wherein the pediococci comprise *Pediococcus acidilactici* strain LMG P-21927.

8. Use according to any one of the preceding claims, wherein the pathogenic bacteria are Gram-negative bacteria.

9. Use according to any one of the preceding claims, wherein the pathogenic bacteria are Gram-positive bacteria.

10. Use according to any one of the preceding claims in combination with one or more further probiotics selected from the group consisting of *Lactobacillus rhamnosus, L. plantarum, L. fermentum, L. acidophilus, L. reuteri, L. casei, L. johnsonii, L. gasseri, L. crispatus, L. helveticus, L. salivarius, L. lactis, L. brevis, L. paracasei, L. sakei, Bifidobacterium animalis, B. lactis, B. adolescentis, B. longum, B. infantis, B. bifidum* and *B. breve*.

11. An isolated pediocin-producing pediococcus characterised by a survival rate as per the Survival Rate Test defined herein of at least 90 %.

12. A pediococcus according to claim 11 wherein the pediococcus is *P. acidilactici* as deposited at BCCM™/LMG under No. LMG P-21927.

13. A health-promoting composition comprising a pediococcus according to claim 11 or 12 as a probiotic component.

14. A health-promoting composition according to claim 13, and further comprising a component selected from the group consisting of pediocin, additional probiotics, prebiotics, immunoglobulins, and mixtures thereof.

15. A method for isolating pediocin-producing pediococci from a substrate, wherein the pediocin-producing pediococci is isolated by using a medium comprising xylose, an antibiotic derived from quinolones and pediocin.

16. A method according to Claim 15, wherein the substrate is human faeces.

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